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Serology of Rubella

Comparison of Fluorescent Antibody, Complement Fixation and Neutralization Tests for Diagnosis of Current Infections and Determination of Sero-immunity

EDWIN H. LENNETTE, M.D., NATHALIE J. SCHMIDT, AND ROBERT L. MAGOFFIN, M.D., *Berkeley*

■ *Neutralization, complement fixation (CF) and indirect fluorescent antibody (FA) assays for rubella virus were compared for sensitivity in the serologic diagnosis of infection, for demonstrating antibody in the sera of infants with suspected rubella syndrome, and in the detection of antibody elicited by past infection (determination of immunity status). The combination of CF and FA tests was shown to be the most useful for serologic diagnosis of infection, largely eliminating the need for the slower and more cumbersome interference neutralization test.*

Neutralizing antibodies were found to appear rapidly in the course of infection, antibodies demonstrable by immunofluorescent staining appeared slightly later, and CF antibodies were rarely demonstrable in sera collected earlier than 14 days after onset of illness. Antibodies detected by all three techniques showed good correlation in infants with clinical evidence of rubella syndrome and corresponding maternal sera. The indirect FA technique compared favorably with the neutralization test for the detection of antibody elicited by past infection (determination of immunity status) and offered distinct advantages in ease of technical performance and more rapid results. In both current and past infections, FA titers tended to be higher than neutralizing antibody titers.

THE MEASUREMENT OF antibody levels to rubella virus has three distinct applications of clinical importance: The serologic confirmation of clinically suspected cases of rubella, the retrospective diagnosis of *in utero* infections of infants, and the determination of immunity status (sero-immunity) of pregnant women exposed to rubella infections. Neutralizing, complement-fixing and indirect fluorescent antibody assays for rubella virus have been performed in this laboratory on serum specimens obtained in such cases since April 1965. This report compares the relative value of the three test procedures for the diagnosis of current infections, describes the antibody levels in infants with clinical evidence of the rubella syndrome, and compares the sensitivity of the three techniques for detection of antibody persisting from past infection—that is, for determination of immunity status.

Materials and Methods

Neutralizing antibody assays. Tests were conducted by the interference technique in tube cultures of the BS-C-1 line of grivet monkey kidney cells. Two-fold dilutions of inactivated (56°C for 30 minutes) serum, ranging from 1:4 through 1:64 were tested against approximately ten 50 per cent interfering doses (InD_{50}) of rubella virus. The inoculated cultures were challenged with 100 TCD_{50} of echovirus type 11 after five days' incubation at 36°C, and results were read two to four days later. Neutralization of the rubella virus was evidenced by a cytopathic effect (CPE) produced by the echovirus, while failure of the test serum to neutralize the rubella virus was indicated by a lack of echovirus CPE (interference).

Complement-fixing (CF) antibody assays. Sera were examined for the presence of rubella CF antibodies by the standard technique of this laboratory adapted to use with the microtiter system.[4] Antigens were prepared from the fluid phase of infected RK-13 (rabbit kidney) cell cultures[7] or BHK-21 (hamster kidney) cell cultures.[8] Infected fluids were concentrated 100-fold by dialysis against polyethylene glycol and the concentrates were treated with one-half volume of anesthetic ether for one hour at room temperature. After centrifugation at 1,500 rpm for 15 minutes the aqueous phase was removed for use as CF antigen. Residual ether was removed by bubbling with nitrogen. Sera (inactivated at 60°C for 30 minutes) were tested at two-fold dilutions ranging from 1:4 through 1:32 against two units of antigen (as determined by block titrations).

Indirect fluorescent antibody (FA) assays. The indirect immunofluorescent staining technique of this laboratory for detection of rubella antibodies is described in detail elsewhere.[5] Briefly, smears of infected BS-C-1 cells (and uninfected cells for control purposes) were fixed with acetone and ringed with quick-drying paint.* Test sera were inactivated at 56° for 30 minutes, and two-fold dilutions ranging from 1:4 through 1:128 were prepared in a 20 per cent suspension of normal beef brain (to reduce nonspecific staining and overstaining). A drop of each serum dilution was applied to a smear of infected cells, and the lower dilutions were also tested against smears of uninfected cells. After incubation at 36°C for 20 minutes in a humidified atmosphere, slides were washed multiple times in phosphate buffered saline solution (PBS), pH 7.2 to 7.4. The combination of specific antibodies in the test serum with rubella virus antigen in the infected cells was detected through the use of fluorescein-labeled anti-human immune globulins prepared in rabbits. The conjugate, diluted appropriately in a 20 per cent suspension of beef brain, was added to the smears and the slides were then incubated at 36°C for 20 minutes. After washing, the smears were mounted in a 25 per cent solution of glycerol in PBS, pH 7.3. The equipment employed in the examination of smears for immunofluorescent staining has been described previously.[5] Antibody titers were expressed in terms of the highest dilution of test serum which gave specific immunofluorescent staining with the rubella-infected cells.

*Manufactured by Tri-Chem, Inc., Belleville, N. J.

TABLE 1.—*Results of Neutralizing, (Neut.), Complement-Fixing (CF) and Fluorescent Antibody (FA) Determinations in Cases of Suspected Rubella.*

Type of test	*Total cases**	*Rise in titer, 4-fold or greater*	*No rise in titer, antibody present*	*No antibody (<1:4)*
CF	114	45	36	33
Neut.	114	37	55	22
FA	114	51	37	26

*Cases with satisfactory paired sera tested; acute-phase serum taken within seven days, convalescent-phase serum taken 14 or more days after onset.

From the Viral and Rickettsial Disease Laboratory, California State Department of Public Health.

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Reprint requests to: Viral and Rickettsial Disease Laboratory, California State Department of Public Health, 2151 Berkeley Way, Berkeley 94704 (Dr. Lennette).

Results

Comparative diagnostic value of neutralization, complement fixation and indirect fluorescent antibody tests. Antibody responses demonstrated by neutralization, CF and FA tests in 114 suspected clinical cases of rubella in which suitable paired serum specimens were obtained are summarized in Table 1. "Paired" specimens were considered satisfactory for diagnosis if the acute-phase serum was collected no later than seven days after onset of illness and the convalescent-phase serum was taken 14 or more days after onset. This group of cases was made up predominantly of pregnant women or their contacts, mostly children, but included a few college students.

The FA test detected significant antibody increases (four-fold or greater rise in titer) in the greatest number of patients, the CF test in slightly fewer, and the neutralization test in the fewest. When the results of the three tests were collated (Table 2), a total of 61 patients showed a diagnostic rise in antibody titer by one or more tests. Of these, only 25 showed positive results in all three tests. In 22 cases, two of the tests were positive, most often by the CF and FA tests, and in 14 instances only a single test was positive. In the latter group, the FA test was positive in seven cases, the CF test in five and the neutralization test in two. Considering the results of any pair of tests independently, slightly more cases were confirmed by the CF and/or FA tests than by other pairs of tests.

TABLE 2.—*Comparative Frequency of Positive Results by Complement Fixation, Neutralization and Fluorescent Antibody Tests in 114 Suspected Cases of Rubella.*

Combinations of tests showing antibody	Number of cases with: Rise in antibody titer, 4-fold or greater	Number of cases with: No rise in titer, antibody present
One or more tests	61	34
All three tests	25	23
Two tests only		
CF and neut.	3	2
CF and FA	12	0
FA and neut	7	3
One test only		
CF	5	0
Neut.	2	4
FA	7	2
Each test independently		
CF	45	25
Neut.	37	32
FA	51	31
Two tests independently		
CF and/or neut	55	32
CF and/or FA	59	30
FA and/or neut	56	34

Code: Neut.=Neutralizing; CF=Complement Fixing; FA=Fluorescent Antibody.

TABLE 3.—*Correlation of Results of Complement Fixation, Neutralization and Fluorescent Antibody Tests in Cases of Suspected Clinical Rubella.*

CF Against Neutralization Test

CF test results	Number of cases	Neutralization test results: Rise in titer, 4-fold or >	No rise, titer 1:4 or >	Negative (<1:4)
Rise in titer, 4-fold or >	45	28	17	0
No rise, titer 1:4 or >	36	6	30	0
Negative <1:4	33	3	8	22
Totals	114	37	55	22

CF Against FA Test

CF test results	Number of cases	FA test results: Rise in titer, 4-fold or >	No rise, titer 1:4 or >	Negative (<1:4)
Rise in titer, 4-fold or >	45	37	8	0
No rise, titer 1:4 or >	36	9	24	3
Negative <1:4	33	5	5	23
Totals	114	51	37	26

FA Against Neutralization Test

FA test results	Number of cases	Neutralization test results: Rise in titer, 4-fold or >	No rise, titer 1:4 or >	Negative (<1:4)
Rise in titer, 4-fold or >	51	32	18	1
No rise, titer 1:4 or >	37	4	31	2
Negative <1:4	26	1	6	19
Totals	114	37	55	22

Code: Neut.=Neutralizing; CF=Complement Fixing; FA=Fluorescent Antibody.

Correlation between neutralizing, complement-fixing and indirect FA responses in clinical rubella. The correlation of positive, inconclusive, and negative findings with the three test procedures in the 114 suspected cases of rubella is shown in Table 3.

The CF and the FA tests each detected significant antibody increases in a number of patients (17 and 18 cases respectively) who showed stationary titers of neutralizing antibody. The converse—neutralizing antibody titer rises missed by CF or FA—occurred much less often (nine and five cases respectively). None of the patients showed CF antibody in the absence of neutralizing

TABLE 4.—*Complement Fixation, Neutralization and Fluorescent Antibody Titers of Patients Showing Significant Antibody Titer Rises in Only a Single Test.*

Patient	Days after onset	Antibody titer CF	Neut.	FA	Patient	Days after onset	Antibody titer CF	Neut.	FA
1.....	6	<4	8	64	8.....	2	<4	8	<4
	34	16	8	128		19	<4	8	64
2.....	5	<4	64	128	9.....	2	<4	8	<4
	21	16	32	128		16	4	8	128
3.....	7	<4	16	64	10.....	3	<4	4	<4
	26	16	32	128		17	4	8	128
4.....	5	<4	64	64	11.....	2	<4	4	4
	21	32	32	64		22	<4	<4	16
5.....	1	<4	16	64	12.....	6	<4	16	<4
	14	8	16	64		15	4	16	16
6.....	3	8	8	64	13.....	1	<4	16	<4
	23	16	64	64		15	4	16	16
7.....	2	<4	<4	<4	14.....	2	<4	<4	<4
	37	4	32	<4		17	4	4	64

Code: Neut.=Neutralizing; CF=Complement Fixing; FA=Fluorescent Antibody.

antibody; FA antibody was present in three cases without neutralizing antibody.

The CF test detected antibody titer increases missed by the FA tests in eight patients; the FA test was positive in 14 patients showing no rise in CF antibody titer, including five with no demonstrable CF antibody.

To illustrate some of the divergent results obtained with the three tests, Table 4 shows the antibody levels of the 14 patients who had significant titer rises demonstrated by a single test. In four of the five patients (Nos. 1 to 5) with titer rises shown only by the CF test, the acute-phase sera were collected five to seven days after onset. Neutralization and FA titers were already relatively high and remained stationary or changed only two-fold, while CF antibody, which appears more slowly (see Table 6), showed significant increases in titer.

Both of the two patients (Nos. 6 and 7) with diagnostically significant rises in antibody level shown only in neutralization tests, had two-fold increases in CF antibody; one showed a stationary FA titer, while the other had no demonstrable fluorescent antibody.

Of the seven patients (Nos. 8 to 14) showing significant titer rises only in FA tests, five had low neutralizing antibody titers which remained stationary or changed only two-fold. Borderline CF titer increases of from less than 1:4 to 1:4 were also demonstrated in five of the patients.

The range of neutralizing, CF and fluorescent antibody titers elicited by rubella infections is shown in Table 5 which correlates the FA titers of convalescent-phase serum specimens with neutralizing and CF antibody titers. These convalescent-phase sera are from the 61 persons shown in Table 2 with serologic evidence of rubella infection by one or more tests. The most frequently-occurring CF titers were 1:8 and 1:16, while

TABLE 5.—*Correlation of Fluorescent Antibody Titers with Neutralization and Complement Fixation Antibody Titers in Convalescent-Phase Serum Specimens.**

CF antibody titer	No. of sera	FA titer <4	4	8	16	32	64	≥128
≥32.....	7	....	....	....	....	....	3	4
16.....	19	....	....	....	1	3	7	8
8.....	22	....	....	....	1	2	9	10
4.....	8	1	....	....	2	....	1	4
<4.....	5	....	....	....	2	1	2	....
Totals	61	1	0	0	6	6	22	26

Neut. antibody titer	No. of sera	FA titer <4	4	8	16	32	64	≥128
≥64.....	8	....	....	....	....	....	3	5
32.....	14	1	....	....	....	2	6	5
16.....	26	....	....	....	3	3	8	12
8.....	8	....	....	....	2	1	3	2
4.....	4	....	....	....	....	....	2	2
<4.....	1	....	....	....	1	....	....	....
Totals	61	1	0	0	6	6	22	26

*Sera from 61 patients showing serologic evidence of rubella infection by one or more test.
Code: Neut.=Neutralizing; CF=Complement Fixing; FA=Fluorescent Antibody.

TABLE 6.—*Antibody Titers Relative to Time After Onset of Illness in 64 Serologically Confirmed Cases of Rubella.*

Antibody titer	Number of sera with antibody shown by CF test, Days after onset—64 cases						Number of sera with antibody shown by Neutralization test, Days after onset—64 cases						Number of sera with antibody shown by FA test, Days after onset—64 cases					
	0-3	4-7	8-13	14-20	21-27	28>	0-3	4-7	8-13	14-20	21-27	28>	0-3	4-7	8-13	14-20	21-27	28>
≥128	....	....	....	....	....	....	....	....	....	....	....	....	....	2	2	17	7	2
64	....	....	....	....	....	....	....	2	....	4	3	1	3	4	....	14	8	1
32	....	....	....	2	4	1	....	1	1	9	4	....	....	1	....	6	....	....
16	....	....	....	13	4	2	6	7	2	19	7	1	....	2	1	4	2	....
8	1	1	1	16	6	....	4	4	1	7	2	1	2	....	....	....	....	....
4	1	....	1	7	1	....	6	2	....	3	....	....	5	1	....	....	....	....
<4	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
<8	41	19	2	3	2	....	27	4	....	....	1	....	33	10	1	....	....	....

Code: Neut.=Neutralizing; CF=Complement Fixing; FA=Fluorescent Antibody.

neutralization titers tended to be one dilution (two-fold) higher, most commonly 1:16 and 1:32. However, in FA tests most of the sera showed titers of 1:64 or 1:128 or greater. A single serum showed neutralizing and CF antibody, but no antibody was demonstrable in the FA test.

Temporal appearance of rubella antibody. Table 6 shows CF, neutralizing and FA titers relative to the time after onset of illness in serologically confirmed cases of rubella. Data were available on 64 patients, including three whose serum specimens were not collected at the recommended times (see footnote, Table 1), but who showed significant rises in rubella antibody titer by one or more tests. This table illustrates the relatively slow development of CF antibody. Only three of the 63 sera collected within seven days after onset showed CF antibody; whereas, neutralizing antibody was demonstrable in 32 of these "acute-phase" sera, including 16 of the 43 sera obtained within three days after onset. The frequency of antibody demonstrable by the FA test within the first week after onset was intermediate (in ten of the 43 sera collected at 0 to 3 days and in ten of the 20 sera collected at 4 to 7 days), probably reflecting a somewhat slower development than neutralizing antibody. However, titers of fluorescent antibody were generally higher than neutralizing antibody levels. Thus, the greater diagnostic value of the CF and FA tests as compared to the neutralization test may be attributable to a slower development of CF and FA antibody, and the higher titers demonstrable by the FA test may account for the ability of this technique to demonstrate diagnostically significant increases in antibody in cases in which CF test shows inconclusive titer increases, for example, less than 1:4 to 1:4 (see Table 4).

Antibody levels in infants with suspected rubella syndrome. Sera from a total of 106 infants with suspected intra-uterine infections were examined by the three antibody assay techniques. In Table 7 it is seen that 89 of the infants showed antibody in one or more tests while 17 did not show antibody by any of the test procedures. Neutralizing and fluorescent antibodies were de-

TABLE 7.—*Comparison of Complement Fixation, Neutralization and Fluorescent Antibody Tests for Demonstration of Antibody to Rubella in Infants with Suspected Intra-uterine Infection.*

Tests showing antibody present	Number of infants by age at time serum was collected					
	Total	0-1 mo.	2-3 mo.	4-6 mo.	over 6 mo.	unknown
Total, any test	89	61	20	4	2	2
All three tests	59	45	10	1	2	1
Two tests only	(24)	(14)	(6)	(3)	....	(1)
CF and neut.	1	....	1	....	....	....
CF and FA	....	....	....	....	....	....
Neut. and FA	23	14	5	3	....	1
One test only	(6)	(2)	(4)	....	....	....
CF	....	....	....	....	....	....
Neut.	2	1	1	....	....	....
FA	4	1	3	....	....	....
Each test independently						
CF	60	45	11	1	2	1
Neut.	85	60	17	4	2	2
FA	86	60	18	4	2	2
All tests negative	17	6	7	2	2	....
Total cases	106	67	27	6	4	2

Code: Neut.=Neutralizing; CF=Complement Fixing; FA=Fluorescent Antibody.

monstrable in more infants than was CF antibody, but of the 89 infants showing neutralizing and/or fluorescent antibody, 60 also had detectable CF antibody.

Table 8 compares antibody titers obtained in the three test systems with sera of 81 infants (0 to 3 months of age) who had antibody demonstrable by one or more tests. A number of sera with high levels of neutralizing or fluorescent antibody had no CF antibody. This may, in part, reflect the rapid decline of the CF antibody titer of the mother, and therefore of passively-acquired CF antibody of the infant, in the interval between infection and delivery and the inability of some infants to produce CF antibody during the first few weeks of life. Also, some of the sera were from infants with questionable symptoms and questionable histories of maternal rubella infections; in these the neutralization and fluorescent antibody titers could well represent residual antibody from an earlier maternal infection with subsequent loss of CF antibody. FA titers of the infants' sera tended to be somewhat higher than the neutralization titers.

TABLE 8.—*Comparison of Rubella Antibody Titers Demonstrated by Complement Fixation, Neutralization and Fluorescent Antibody Tests on Sera of 81 Infants (0-3 Months of Age) with Suspected Intra-uterine Infections.**

Neut. titer	No. of infants	CF antibody titer				
		<4	4	8	16	≥32
≥64	23	2	3	2	12	4
32	23	6	5	5	4	3
16	15	6	3	4	2	....
8	8	4	2	2	....	....
4	8	3	5	....	....	....
<4	4	4	....	....	....	....
Totals	81	25	18	13	18	7

FA titer	No. of infants	CF antibody titer				
		<4	4	8	16	≥32
≥128	28	6	4	4	8	6
64	17	4	....	5	8	....
32	13	4	6	1	1	1
16	11	3	5	3	....	....
8	6	3	2	....	1	....
4	3	3	....	....	....	....
<4	3	2	1	....	....	....
Totals	81	25	18	13	18	7

Neut. titer	No. of infants	FA titer						
		<4	4	8	16	32	64	≥128
≥64	23	....	....	1	....	2	4	16
32	23	1	....	....	4	5	7	6
16	15	....	....	3	2	3	4	3
8	8	1	3	....	2	....	....	2
4	8	1	....	....	2	3	1	1
<4	4	....	....	2	1	....	1	....
Totals	81	3	3	6	11	13	17	28

*Infants having antibody demonstrated by one or more tests.

TABLE 9.—*Correlation of Rubella Antibody Titers Demonstrated by Complement Fixation, Neutralization and Fluorescent Antibody Tests on Maternal and Infant Sera in 30 Cases of Suspected Intra-uterine Infection.**

CF Test

Mother's titer	No. of cases	Baby's antibody titer					
		<4	4	8	16	32	64
64	....	....	....	....	....	....	....
32	3	....	....	1	1	1	....
16	4	1	....	1	2	....	....
8	7	1	....	3	2	1	....
4	7	1	2	3	1	....	....
<4	9	9	....	....	....	....	....
Totals	30	12	2	8	6	2	....

Neutralization Test

Mother's titer	No. of cases	Baby's antibody titer					
		<4	4	8	16	32	64
64	5	....	....	....	....	1	4
32	5	....	....	1	1	2	1
16	9	....	1	....	3	2	3
8	4	....	1	2	....	1	....
4	3	2	1	....	....	....	....
<4	4	2	....	1	1	....	....
Totals	30	4	3	4	5	6	8

FA Test

Mother's titer	No. of cases	Baby's antibody titer						
		<4	4	8	16	32	64	≥128
≥128	6	....	....	....	1	....	....	5
64	12	....	1	....	....	3	4	4
32	4	....	....	....	....	1	3	....
16	1	....	....	....	1	....	....	....
8	1	....	....	....	1	....	....	....
4	1	....	....	....	1	....	....	....
<4	5	3	2	....	....	....	....	....
Totals	30	3	3	....	4	4	7	9

*Sera obtained within one month of birth from mother and infant.

Code: Neut.=Neutralizing; CF=Complement Fixing; FA=Fluorescent Antibody.

In Table 9 neutralizing, CF and fluorescent antibody titers of infants less than one month of age are compared with antibody titers of their mothers' sera taken at, or about, the same time. Correlation of all three types of antibody is seen to be fairly good. In both the mothers' and infants' sera CF antibody was less often demonstrable than fluorescent or neutralizing antibody but in some instances the infants' CF antibody titers exceeded those of their mothers.

It has been shown that rubella neutralizing antibody persists in congenitally-infected infants long after maternally-acquired antibody normally

is lost, possibly for life.[3,9] However, the neutralizing antibody has been found to undergo changes in physicochemical properties, consisting largely of 7S globulins at birth, presumably of maternal origin; these antibodies diminish and are replaced by 19S antibody which reaches maximum levels at four to seven months. The 19S antibody is then replaced by 7S antibody which may persist throughout life.[1,2] It would appear that information of some diagnostic value might be gained by the examination of a serum specimen taken shortly after birth together with one taken after maternal antibody would normally have disappeared. If antibody is present in the first specimen but absent or declining in the second, it is probably of maternal origin, but if antibody persists after the time at which maternal antibody normally is lost, it is likely to have been produced by the infant in response to a chronic, congenitally-acquired infection.

Dual serum specimens were examined from 20 infants with suspected rubella syndrome; three of these infants had no antibody in either specimen and in six cases the specimens were collected too close together for a comparison of antibody titers to have meaning. Results of antibody assays and virus isolation attempts on the 11 infants with suitably-spaced serum specimens are presented in Table 10. Five of the infants (Nos. 1 to 5) yielded virus in their throat washings, and it is seen that their antibody levels remained stationary (or changed no more than two-fold) for one to four months after birth. Patient No. 6 had a negative virus isolation attempt, but the persistence of all three kinds of antibody suggests a congenital infection. Neutralizing and fluorescent antibody levels of the first six patients were generally higher than those for patients Nos. 7 to 11, whose antibody appears to have been solely of maternal origin since it had disappeared or decreased sharply by the time the second specimen was taken. The results of tests on this small number of infants indicate that antibody assays on suitably spaced serum specimens may aid in the retrospective diagnosis of infection with rubella virus *in utero.*

TABLE 10.—*Results of Virus Isolation Attempts and Antibody Assays on Sera of Infants with Suspected Rubella Syndrome.*

Patient	Sex	Virus isol.	Time after birth	Antibody titer CF	Neut.	FA
1	F	+	4 days	8	16	64
			1 month	4	8	64
2	F	+	2 months	<4	32	128
			4 months	<4	64	128
3	F	+	1 day	4	16	128
			1 month	4	32	128
4	F	+	2 weeks	<4	16	128
			1 month	4	32	128
5	M	+	7 weeks	16	64	128
			3.5 months	8	64	128
6	M	0	2 days	16	64	128
			2 months	16	32	128
7	F	0	3 months	<4	16	8
			4 months	<4	8	<4
8	M	0	4 days	4	32	16
			2.5 months	4	4	<4
9	M	0	1 month	4	8	16
			4 months	<4	4	<4
10	F	0	7 weeks	8	8	16
			6 months	<4	<4	<4
11	F	0	2 weeks	16	64	128
			6 weeks	<4	4	32

Code: Neut.=Neutralizing; CF=Complement Fixing; FA=Fluorescent Antibody.

TABLE 11.—*Antibody from Past Rubella Infections Detected by Complement Fixation, Neutralization and Indirect Fluorescent Antibody Tests. Sera from 117 Persons Obtained at Time of Exposure.*

Antibody detected by:	Number of persons with detectable antibody
Total, any test	96
All three tests	69
Two tests only	(16)
CF and neut.	1
CF and FA	0
Neut. and FA	15
One test only	(11)
CF	1
Neut.	7
FA	3
Each test independently	
CF	71
Neut.	92
FA	87
All tests negative	21
Total persons tested	117

Code: Neut.=Neutralizing; CF=Complement Fixing; FA=Fluorescent Antibody.

Comparative sensitivity of neutralization, FA and CF tests for demonstration of antibody elicited by past infections. To compare the sensitivity of the three tests for detection of antibody elicited by past infection, sera were examined from individuals who had been exposed to rubella but had no clinical evidence of infection. Antibodies in these sera, collected within a few days of exposure, were considered to have been produced in response to past infection. In Table 11, it is seen that of 117 post-exposure sera examined, 96 showed antibody by one or more test procedures, while 21 did not show antibody in any test. The neutralization test, which detected antibody in 92 sera, was the most sensitive; the FA test was only

slightly less sensitive, detecting antibody in 87 sera. The CF test detected antibody in approximately 74 per cent of the sera showing neutralizing and/or fluorescent antibody. The single individual who had rubella antibody demonstrable only in the CF test had a titer of 1:4; this is the only serum specimen out of hundreds tested which showed CF antibody in the absence of neutralizing or fluorescent antibody.

The sensitivity of the neutralization and FA tests for detecting antibody from past infections is further compared in Table 12, which shows the antibody titers demonstrated by each test in two groups of persons. The first group consisted of 93 persons with antibody demonstrable in sera collected at the time of exposure, and the second of 33 who had illnesses clinically suspicious of rubella but who had antibody in their acute-phase serum specimens and did not show increases in antibody titer. These persons are considered to have had past, but not current, infections with rubella virus. In both groups of sera, the FA titers tended to be higher than neutralizing antibody titers, but in each group there were a few more persons with antibody detected by the neutralization test only, than by the FA test only.

Discussion

The clinical diagnosis of rubella is often equivocal; hence, a reliable serologic test to confirm suspected infections in pregnant women offers the physician a more secure basis for decisions regarding the management of the pregnancy. Fairly often, however, the time required to obtain the laboratory result seriously limits its usefulness to the physician. The neutralization test, while providing a generally accepted standard against which other serologic tests may be compared for sensitivity and specificity, requires at least a week, often ten to fourteen days, to perform after the test sera are in hand and previously prepared cell cultures are ready for inoculation. The CF and FA tests can be completed in one or two days and, equally important, are more adaptable for setting up new test-runs several times weekly. Thus, if equally reliable, the CF and FA methods both possess distinct advantages in time and ease of performance over the more cumbersome neutralization procedure.

In these studies the CF and indirect FA tests were about equally useful for the serologic diagnosis of current rubella virus infections. The use of both tests provided the most effective diagnostic approach and this is the current practice in this laboratory. The FA test, in our hands, is more sensitive than the CF test in detecting specific antibody, and in certain instances it may demonstrate a significant rise in antibody titer while the CF test remains negative or shows an equivocal increase in titer from less than 1:4 to 1:4. On the

TABLE 12.—*Correlation of Fluorescent Antibody and Neutralization Antibody Titers of Individuals with Past Rubella Virus Infections.*

A. 93 Individuals with Antibody at Time of Exposure

Neut. titer	No. of sera	FA titer						
		<4	4	8	16	32	64	≥128
≥64	11	1	....	....	1	4	3	2
32	20	....	....	....	2	5	9	4
16	25	1	1	2	6	7	4	4
8	21	3	....	2	6	6	4	....
4	13	2	1	6	1	3	....	....
<4	3	....	1	....	....	2	....	....
Totals	93	7	3	10	16	27	20	10

B. 33 Patients with Suspected Clinical Rubella who Showed Antibody in Acute-phase Illness Specimens

Neut. titer	No. of sera	FA titer						
		<4	4	8	16	32	64	≥128
≥64	3	....	....	....	1	....	1	1
32	6	1	....	....	1	3	1	....
16	12	1	....	2	....	5	1	3
8	7	2	....	1	....	3	1	....
4	3	1	....	1	1	....	....	....
<4	2	....	....	1	....	....	1	....
Totals	33	5	0	5	3	11	5	4

Code: Neut.=Neutralizing; CF=Complement Fixing; FA=Fluorescent Antibody.

other hand, CF antibody is slower to appear than antibody detectable by immunofluorescent staining; hence, in some cases in which the acute-phase serum specimen is not collected promptly, fluorescent antibody may have already reached high levels and not increase further, while CF antibody shows a significant titer rise. As neutralizing antibody usually appears earlier and reaches maximal titers sooner than either FA or CF antibody, neutralization tests have rarely demonstrated significant titer rises missed by the other two tests.

Sever and coworkers[6] have reported a less favorable experience with FA tests. On three of the four examples of antibody responses in rubella given in their report, neutralizing and CF antibody titers showed significant increases during the course of the illness, but FA titers were at high levels on the first or second day of the rash, and significant increases in FA titer could not be demonstrated. The first specimens on their patients were taken *before* infection, and thus seroconversion was demonstrated by all tests. In our studies, however, fluorescent antibody appeared slightly later than neutralizing antibody, so that significant increases were demonstrable by the FA test in a number of patients whose neutralizing antibody titers were already elevated in the acute-phase specimens (this was seen in 18 of 51 cases confirmed by FA tests).

Studies on the small number of infants in our series with suitably-spaced serum specimens indicate that antibody assays on a serum specimen taken soon after birth together with one taken several months later, when maternal antibody would normally have disappeared, may aid in the diagnosis of congenitally-acquired infections. In infants with chronic infections acquired *in utero,* antibody levels persist. The FA or neutralization test would appear to be most useful for this purpose, since in some instances CF antibody was absent or at low levels. Sever and coworkers[6] noted that the CF titers of infants infected *in utero* decline between one to five months following birth, but that after the fifth month CF antibody levels increase, frequently exceeding those seen at the time of birth. None of the infants' sera examined in our studies were collected at appropriate times to demonstrate such a decline and reappearance of CF antibody.

From these investigations it would appear that the FA test is adequately sensitive for detection of antibody elicited by past infection—that is, for determination of immunity status. Sera taken at the time of exposure showed neutralizing antibody without corresponding fluorescent antibody slightly more often than *vice versa,* but on the other hand FA titers tended to be higher than neutralizing antibody titers. While more extensive information on the persistence of fluorescent antibody as compared with that of neutralizing antibody is needed, from the data thus far obtained we believe the technical advantages of the FA method outweigh its slightly lesser sensitivity. FA tests are less cumbersome and expensive to perform than are interference neutralizing tests, they are beset with fewer variables, endpoints are more reproducible and results can be obtained more rapidly. While CF antibody was not demonstrable in about 25 per cent of post-exposure sera showing antibody in neutralization and/or FA tests, the CF test can be useful for rapid screening for the determination of immunity status, leaving the minority of sera failing to show CF antibody to be further examined in FA or neutralization tests. The CF test proved to be highly specific; only a single serum specimen out of hundreds examined had CF antibody (a low titer of 1:4) in the absence of neutralizing or fluorescent antibody.

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